Synopsis of the Thesis entitled

QUANTITATIVE DETERMINATION OF ACTIVE PHARMACEUTICAL INGREDIENTS, RELATED SUBSTANCES AND ORGANIC & POLYMORPHIC IMPURITIES IN PHARMACEUTICAL FORMULATIONS BY LIQUID CHROMATOGRAPHY AND NEAR INFRARED SPECTROSCOPY

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SYNOPSIS

Pharmaceutical research in the last few decades has resulted in the launch of numerous drugs. The advanced and extremely potent drugs have found their applicability in treating various types of ailments. Because these drug substances are highly potent, a few of the impurities associated with these are also found to be very toxic. If these impurities are passed on to the drug substance or drug product can lead to serious toxicological consequences. The presence or conversion to a different polymorph can bring about differences in solubility and bioavailability. The legal implications also significantly impact the drug development and stability aspects of the drug.

The need to develop new analytical methods with extremely high sensitivity together with precision and accuracy thus became mandatory. These methods are needed for assurance of quality, safety and efficacy of drugs and pharmaceuticals. The need for these advanced methods is not only because of the safety but also because of the increasingly vigilant and stringent regulatory requirements. The analytical methods assume great importance due to i) development of new drugs ii) continuous improvements in manufacturing processes for existing drugs and iii) revision of threshold limits for individual and total impurities of drugs by regulatory authorities. This is the basis for the present research work. The methods developed as a part of this thesis fulfill all the above mentioned requirements. All the methods described in the thesis are simple, novel, fast, reliable and validated. The thesis comprises of six chapters as described below.

Chapter 1

GENERAL INTRODUCTION

Chapter 1 gives a brief introduction to different types of analytical methods with special reference to pharmaceutical applications. The basic introduction of various types of pharmaceutical dosage forms, their manufacturing processes and different types of ingredients used in formulations is detailed. The chapter next describes the various aspects of the combination products, their therapeutic advantages and utility. The risks involved in the combining these drugs is also explained in detail. The exemplified

explanation of the drug interactions with those of the co-administered drugs has been detailed along with possible chemical interactions.

The interactions between drugs can lead to various degradations because of their chemical incompatibilities. These degradations can happen via Oxidation, Hydrolysis, Polymerization, Isomerization, Decarboxylation, Dehydration, polymorphism and Formation of insoluble complexes.

A brief description on the principles of HPLC, UPLC and Near Infrared spectroscopy along with their instrumentation and working principles is also a part of this chapter. The application of various instruments in determination of polymorphism in polymorphic solids is discussed. The chapter concludes with detailed discussion on the practical approach towards method development and validation by liquid chromatography.

Chapter-2

Method Development for Related substances of Atorvastatin & Clopidogrel in combination by UPLC

This chapter describes a reversed phase Ultra Performance liquid Chromatographic method for determination of related substances in combination products of Atorvastatin and Clopidogrel in pharmaceutical dosage forms. **The method has a unique advantage of separating 13 peaks within an extremely short run time of 12 minutes.**

The API characterization data for Atorvastatin and Clopidogrel is presented along with the impurity details in this chapter.

The step wise logical scientific method development has been described along with the reasoning. The optimized UPLC conditions are as follows; Buffer:10 mM KH_2PO_4, 1ml of TEA and pH to 2.50, adjusted with OPA, and 1gm/1000ml of 1-Octane Sulphonic acid Sodium salt as the mobile phase-A, mobile phase-B being Buffer: Acetonitrile::Methanol :: 36:154:10 v/v/v

The Chromatographic Conditions: are, Flow rate -0.7 ml/min, Column temperature-30°C, detection Wavelength at220 nm, Inj Volume of 2µL. Column used is Agilent Eclipse plus C18 RRHD, 50X2.1mm, 1.8µm. The gradient elution pattern presented with respect to time Vs % mobile phase B is as follows 0.01-25, 0.5-25, 3.0-50, 7.0- 50, 10.0-

80, 10.5-80,10.7-25,12.0-25. Forced degradation studies are carried out by stressing at variety of conditions. All the degradant peaks are well separated from the main peak and the impurity peaks. The method is validated with respect to precision (Repeatability of all the 13 peaks) and found to be precise. The accuracy is carried out on 5 levels from 50 % to 200% of the specification limit and the recoveries of all the peaks are within acceptable limits. The linearity is carried out on 6 levels from 50 % to 200% of the specification limit. The correlation coefficient is found to be more than 0.998 for all the 13 peaks. Limit of detection (LOD) and Limit of quantitation (LOQ) results demonstrated the high sensitivity of the method.

The method is found to be specific, precise, linear and accurate in the range of its intended application. This method is suitable for use in routine analysis in any quality control laboratory and if applied will prove to be extremely beneficial for the organization and the end user i.e. the patient

Chapter 3

Method Development and validation for Related substances of Bisoprolol & Hydrochlorothiazide in combination

This chapter describes a reversed phase Ultra Performance liquid Chromatographic method for determination of related substances in combination products of Bisoprolol and Hydrochlorothiazide in pharmaceutical dosage forms. **The method has a unique advantage of separating 8 peaks within an extremely short run time of 10 minutes.**

The API characterization data is presented along with the impurity details in this chapter. The step wise logical scientific method development has been described along with the reasoning. The optimized UPLC conditions are as follows Buffer: 2mL of 85%v/v OPA in 1000mL of water. The buffer is used as mobile phase-A. Mobile phase-B being 2mL of 85%v/v OPA in Acetonitrile

The Chromatographic Conditions: are Flow rate -0.2 ml/min, detection Wavelength at 220 nm, Inj Volume of 1µL, Columns used is Acquity UPLC BEH C18 , 100X2.1 mm,1.7µm. The gradient elution pattern presented with respect to time Vs % mobile phase B is as follows 0.01-5, 4.5-18, 7.6-45, 8.2- 60, 8.5.0-60, 8.8-5,10.0-5.

Forced degradation studies are carried out by stressing at variety of conditions. All the degradant peaks are well separated from both the principle peaks and the impurity peaks. The method is validated with respect to precision (Repeatability of all the 8 peaks) and found to be precise. The accuracy is carried out on 7 levels from 12 % to 150% of the specification limit and the recoveries of all the peaks are within acceptable limits. The linearity is carried out on 8 leves from LOQ to 150% of the specification limit. The coorelation coefficient is found to be more than 0.998 for all the 8 peaks. Limit of detection (LOD) and Limit of quantitation (LOQ) results demonstrated the extremely high sensitivity of the method.

The method is found to be specific, precise, linear and accurate in the range of its intended application. This method is suitable for use in routine analysis in any quality control laboratory and if applied will prove to be extremely beneficial for the organization and the end user i.e. the patient.

Chapter-4

Method Development and validation for related substances of Omeprazole in API and formulations by UPLC.

This chapter describes a reversed phase Ultra Performance liquid Chromatographic method for determination of related substances in drug products of Omeprazole in pharmaceutical dosage forms. **The method has a unique advantage of separating 5 peaks within an extremely short run time of 3 minutes.**

The API characterization data is presented along with the impurity details in this chapter.

The step wise logical scientific method development has been described along with the reasoning has been described. The optimized UPLC conditions are as follows

The buffer used as mobile phase A is as follows: The Mobile phase A is 1.40 gms of sodium dihydrogen phosphate in 1000 ml of water , 1ml of triethylamine, pH to 7.5 adjust with dilute OPA. The mobile phase B being Acetonitrile and Methanol in the ratio of 90:10 v/v.

The Chromatographic Conditions are: Flow rate -0.6 ml/min, detection Wavelength at 280 nm, Inj Volume of 8µL, Columns used is HSS T3 100 x 2.1 mm, 1.8 µ maintained

at 50˚C. The gradient elution pattern presented with respect to time Vs % mobile phase B is as follows 0.01-25, 0.5-30, 1.5-45, 2.0- 45, 2.3-90, 2.6-90,2.7-25, 3.0- 25.

Forced degradation studies are carried out by stressing at variety of conditions. All the degradant peaks are well separated from the main peak and the impurity peaks. The method is validated with respect to precision (Repeatability of all the 6 peaks) and found to be precise (%RSD <0.55). The accuracy is carried out on 6 levels from 50% to 300% of the specification limit and the recoveries of all the peaks are within the acceptable limits. The linearity is carried out on 8 levels from LOQ to 300% of the specification limit. The coorelation coefficient is found to be more than 0.999 for all the 6 peaks. Limit of detection (LOD) and Limit of quantitation (LOQ) results demonstrated the extremely high sensitivity of the method.

The method is found to be specific, precise, linear and accurate in the range of its intended application. This method is suitable for use in routine analysis in any quality control laboratory and if applied will prove to be extremely beneficial for the organization and the end user i.e. the patient.

Chapter-5

Method Development and validation for Related substances of Montelukast & Levocetirizine in combination by HPLC

This chapter describes a reversed phase High Performance liquid Chromatographic method for determination of related substances in combination products of Montelukast and Cetirizine in pharmaceutical dosage forms. The method describes separations 13 peaks with a run time of 65 minutes.

The API characterization data is presented along with the impurity details in this chapter.

The step wise logical scientific method development has been described along with the reasoning. The optimized HPLC conditions are as follows

The buffer used as mobile phase A is as follows: 1ml of 85% OPA in 1000ml HPLC grade water. The mobile phase B being Acetonitrile::Methanol :: 90:15. The Chromatographic Conditions are: Flow rate -1.5 ml/min, detection Wavelength at 225 nm, Inj Volume of 30µL, Columns used is Waters symmetry shield RP18, 250X4.6mm,

5µm. The gradient elution pattern presented with respect to time Vs % mobile phase B is as follows 0.01-25, 10-30, 15-35, 20- 50, 30-65, 40-80,50-100, 55-100,58-25, 65-25.

Forced degradation studies are carried out by stressing at variety of conditions. All the degradant peaks are well separated from the main peak and the impurity peaks. The method is validated with respect to precision (Repeatability of all the 13 peaks) and found to be precise. The accuracy is carried out on 6 levels from 50% to 300% of the specification limit and the recoveries of all the peaks are within acceptable limits. The linearity is carried out on 6 levels from 50% to 300% of the specification limit. The correlation coefficient is found to be more than 0.998 for all the 13 peaks. Limit of detection (LOD) and Limit of quantitation (LOQ) results demonstrated the extremely high sensitivity of the method.

The method is found to be specific, precise, linear and accurate in the range of its intended application. This method is suitable for use in routine analysis in any quality control laboratory and if applied will prove to be extremely beneficial for the organization and the end user i.e. the patient.

Chapter 6

Polymorphic purity determination of Sertraline Hydrochloride by NIR

This chapter describes the importance of Near infrared spectroscopy in determination of polymorphism in pharmaceutical solids. The basics and spectral aspects of Near infrared spectroscopy is described in the initial part along with the description on the NIR spectra, spectral derivatives, and other statistical methods of data interpretation.

The chapter provides a brief introduction to Sertraline HCl. The NIR spectra of Polymorph 1 and polymorph 2 (Form I and Form II) are depicted along with the minute details there in. To develop the method, analysis of pure samples of Form I and Form II, spiked samples of Form I in Form II are performed with serial dilution method and scanned by NIR. Individual samples are prepared containing 0.5%, 1.0%, 2.0%, 5.0%, 10.0% and 20% w/w of form I in Form II and analysed by means of NIR.

The spectral data obtained with these samples is formatted by statistical tools such as multivariate analysis i.e. Conformity index and quantitative model. The results thus

obtained are used to calculate the correlation coefficient which confirmed that there is absolute agreement between the true and the predicted values provided by the software.

All these spiked samples are also analysed by Differential scanning calorimeter (DSC) and attempts are made to correlate the results with those obtained form NIR and found that DSC is not found to be a suitable technique for determination of polymorphic purity of Sertraline HCl.

The polymorphic purity determination method by NIR spectroscopy is thus developed as the only simple and reliable method to determine even very low levels of polymorphic impurity i.e.as low as 0.5%. Both the Conformity index method as well as the quant model can be used as efficient modes to determine the polymorphic purity for Sertraline HCl by NIR.